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Streptolysin O: Suppression of Its Antigenicity by Lipids Extracted from Skin[1] (38070)

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Antibodies to streptolysin O, an extracellular product of Group A streptococci, have been the most frequently utilized serologic indicator of human infection with these organisms. However, data from several laboratories, including a prospective study from our own laboratory, have indicated that the immune response to streptolysin O is feeble following streptococcal impetigo when compared with that following streptococcal upper respiratory tract infection (1–4). The capacity to respond vigorously to other streptococcal antigens—as demonstrated by markedly increasing antistreptococcal desoxyribonuclease B titers—suggested that this suppression might be specific for streptolysin O (4). This weak response to streptolysin O did not appear to be the result of diminished production of the antigen by Group A streptococci; the strains of Group A streptococci recovered from different body sites of patients in this latter study did not differ appreciably in their ability to produce streptolysin O *in vitro* (4).

Streptolysin O has several toxic biological properties including cardiotoxicity and an ability to hemolyze erythrocytes. In 1939 Todd and Hewitt showed that cholesterol inhibits the hemolytic capacity of streptolysin O and also is able to protect mice against lethal intravenous doses of streptolysin O (5). Since cholesterol and other similar sterol compounds are present in abundance in skin (6, 7), we hypothesized that the poor antibody response to streptolysin O might be related to the site of infection per se, i.e., that cholesterol and/or other related lipid compounds present in the skin might block the antigenicity as well as the hemolytic capacity of this specific streptococcal extracellular product.

Materials and Methods. Lipid extracts of skin were prepared as follows: Dermis and epidermis were harvested from albino New Zealand rabbits and the hair and subcutaneous adipose tissue were carefully removed. The skin was cut into small pieces and 20 g of skin were placed into the mixing cup of a Sorvall Omni-Mixer (Ivan Sorvall, Inc., Newton, Connecticut); a mixture of chloroform and methanol (2:1 ratio) was added to the cup. The skin was ground for 20 min and the supernatant solution containing lipid extract was removed and evaporated slowly. The residual lipid material was suspended in absolute methanol, dissolved with heat, and the solution was poured into boiling sterile physiological saline, allowing the methanol to boil off. The resulting suspension of lipid material in saline was centrifuged slowly to remove large particulate matter. At each point in the procedure the material was cultured aerobically and anaerobically to monitor for sterility. Preliminary analysis[2] of similar undiluted lipid extracts of rabbit

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[2] These analyses were kindly performed by Drs. Don Downing and John Strauss, Dept. of Dermatology, Boston University School of Medicine, Boston, Massachusetts.

dermis and epidermis suspended in saline revealed a cholesterol concentration of 0.67 mg per ml.

To determine whether lipids extracted from rabbit dermis and epidermis possessed the ability to block the hemolytic capacity of streptolysin O, ten units of reduced streptolysin O (Difco Company, Detroit, Michigan) were added to each of 10 test tubes. One ml of successive two-fold (undiluted, 1:2, 1:4, ... 1:512) dilutions of the saline suspension of the lipid extract of skin was added to each tube and the mixture was incubated for 20 min. One-half ml of washed 5% rabbit erythrocytes was then added to each tube bringing the test mixture in each tube to a total volume of 3 ml. The mixture was again incubated for 20 min, centrifuged, and the supernatant examined for evidence of hemolysis.

In an experiment to test the ability of lipid extracts of dermis and epidermis to suppress the antigenicity of streptolysin O, 30 albino New Zealand rabbits were divided into 2 groups. The first group (15 rabbits) was immunized intravenously twice weekly for 10 successive weeks with 33 units of reduced streptolysin O per injection (a previously determined nonlethal dose for these rabbits) diluted in sterile physiological saline to a final volume of 2 cc. The second group was immunized according to the same schedule with a mixture of streptolysin O and skin lipid extract in a ratio previously shown to block the hemolytic capacity of this amount of streptolysin O *in vitro:* 33 units of streptolysin O diluted in 0.5 cc of saline mixed with skin lipid extract suspended in 1.5 cc of saline (total cholesterol content approximately 1.0 mg). All rabbits were bled prior to immunization and at intervals up to 11 wk following the initiation of immunization with streptolysin O. During the 11 wk period, 5 of the animals died from or were sacrificed due to causes unrelated to immunization. All sera collected from each animal during the experiment were analyzed simultaneously and blindly for the presence of antistreptolysin O (8). The geometric mean antibody titers were determined for each group at each bleeding and the mean titers for the two groups were statistically compared.

Results. Figure 1 shows the results of a typical *in vitro* experiment demonstrating the ability of the skin lipid extract to block hemolysis of the erythrocytes by streptolysin O. The skin extract proved to be a potent inhibitor of the hemolytic capacity of streptolysin O, even in relatively dilute concentrations.

Figure 2 illustrates the individual and the geometric mean antistreptolysin O titers for the 2 groups of rabbits at intervals before, during and after immunization with streptolysin O. No antibodies to streptolysin O

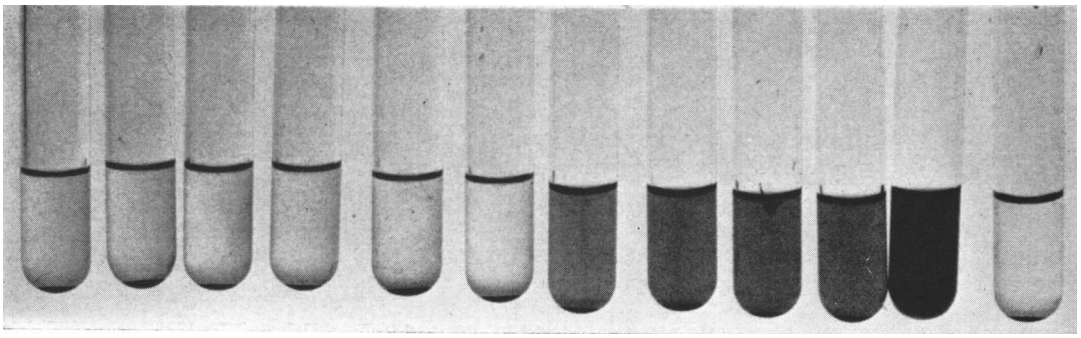

FIG. 1. The ability of lipids extracted from rabbit skin to inhibit the hemolytic capacity of streptolysin O is shown. In the 10 tubes on the left the concentration of skin lipid extract decreases progressively from left to right (see text); the amount of streptolysin O is kept constant. On the right are the two controls: one, in which only streptolysin O is added to red cells, shows complete hemolysis and the other, consisting of only lipid extract and red cells, shows no hemolysis.

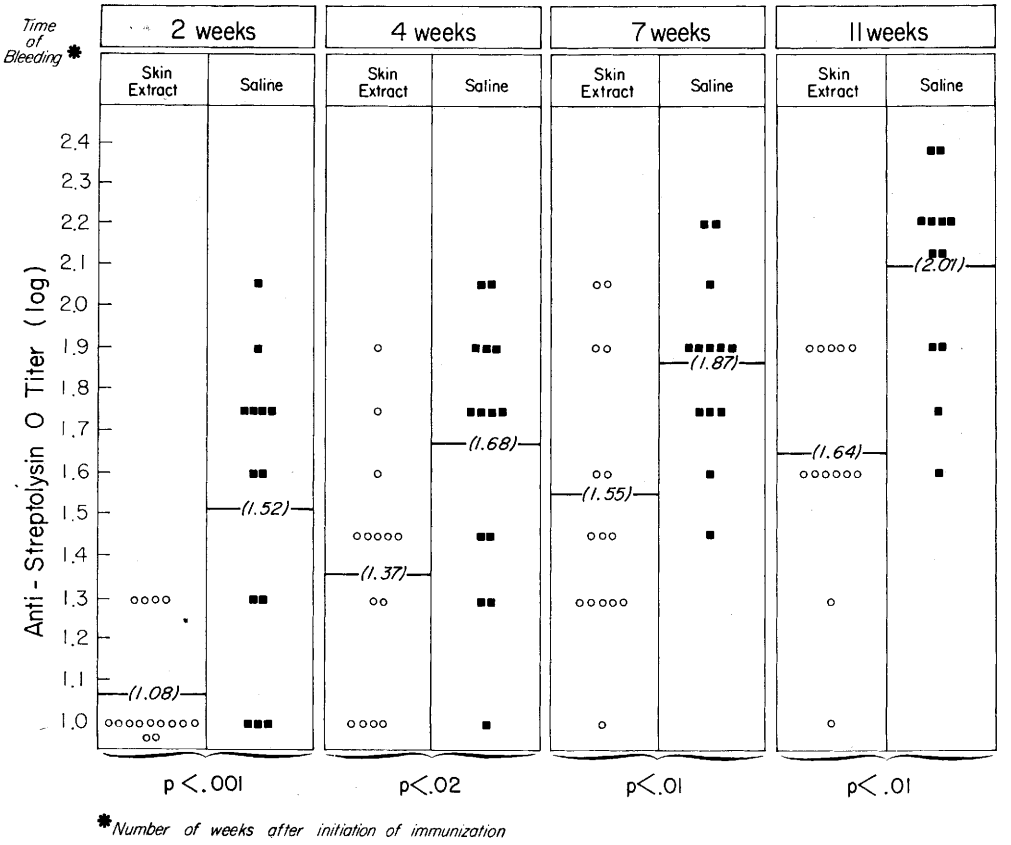

FIG. 2. This scattergram compares the antistreptolysin O titers in 2 groups of rabbits: In the left of each pair of panels, those immunized with a mixture of streptolysin O and lipids extracted from skin and, in the right, those immunized with streptolysin O and saline (see text).

were detectable in animals from either group prior to immunization. For each of the subsequent bleedings, both during and following the immunization period, the geometric mean of the antistreptolysin O titer (shown by the horizontal lines) was lower for the group of animals receiving streptolysin O with skin lipid extract than that for the control group which received the antigen with only saline. The differences were highly statistically significant for each bleeding. There was slight overlap in the distribution of individual titers in the 2 groups, but the distributions generally reflect the differences in the geometric mean titers.

Discussion. These findings provide evidence that the immune response to streptolysin O is inhibited by lipid compounds present in dermis and epidermis. Although cholesterol has been shown to be able to block both the hemolytic and cardiotoxic effects of streptolysin O (5) it is probable that other related lipid compounds may also possess the ability to interfere with these biologic properties as well as with the antigenicity of streptolysin O. Studies are presently underway to characterize further the lipids present in the extract of dermis and epidermis and to determine their role in the suppression of the antigenicity of streptolysin O.

These data from this study also offer an explanation for the feeble response to streptolysin O following streptococcal impetigo. The exact mechanism(s) responsible for this suppression of the antigenicity is not clear. By binding to streptolysin O lipids may block or modify an antigenic

site(s) on the streptolysin O molecule, thereby resulting in a lower antistreptolysin O response.

These observations are of particular interest since streptolysin O is known to be toxic for heart tissue and a possible role for this antigen in the pathogenesis of rheumatic fever has been postulated (9). Thompson and colleagues have found mammalian myocardial cells in tissue culture to be rapidly damaged by streptolysin O (10). Ginsburg has suggested that this antigen may, by causing tissue injury in the heart, prepare the tissue for subsequent localization of streptococcal cell wall components and/or immunologic injury (11). Perhaps modification of the toxicity of or the immune response to streptolysin O is responsible for the clinical observation that rheumatic fever does not follow streptococcal infection of the skin (12).

Summary. Chloroform:methanol extractable lipid(s) from rabbit dermis and epidermis has the ability to prevent hemolysis of erythrocytes by streptolysin O, an extracellular antigen of Group A streptococci. The data from this study also indicate that these same lipid preparations are able to suppress the immune response to this streptococcal antigen. These experimental data appear to provide a logical explanation for the epidemiologic finding that the antistreptolysin O response is feeble following streptococcal impetigo. They may also bear on the clinical observation that rheumatic fever fails to develop after Group A streptococcal infections of the skin.

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